LA-UR-12-26909

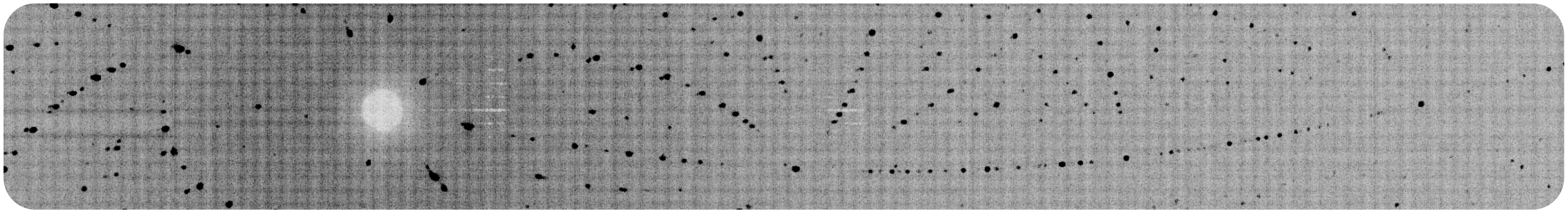
Approved for public release; distribution is unlimited.

Title: Applications of Neutron Diffraction Protein Crystallography

Author(s): Gonzalez, Javier M.

Intended for: Invited speaker at Institute of Molecular and Cell Biology of Rosario, Argentina.

Applications of Neutron Diffraction Protein Crystallography



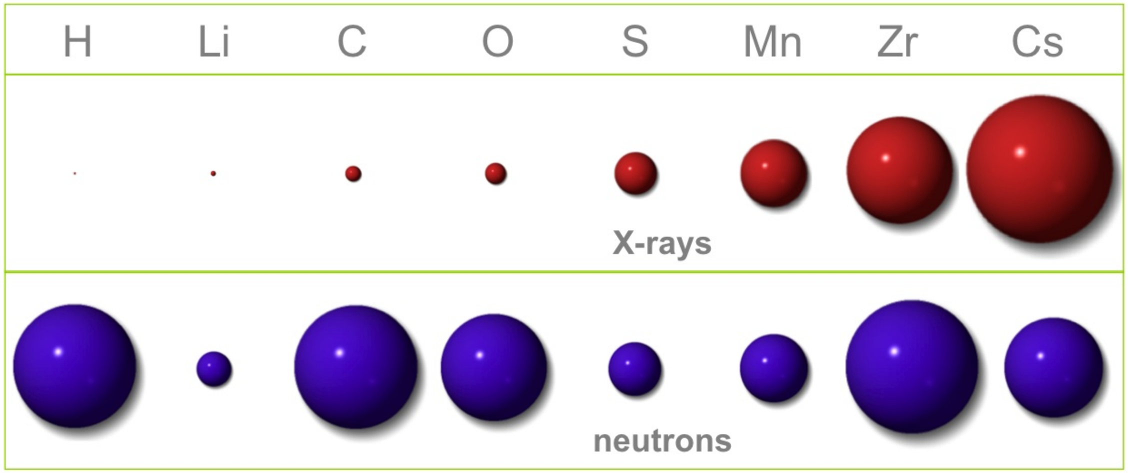
IBR-CONICET

Rosario, Argentina. 12-17-2012

Javier M. González
Bioscience Division, PCS, LANL.

LA-UR-XX-XXXXX

Neutron vs. X-ray scattering



Atom	Coherent Scattering	Incoherent Scattering
H	-3.7	80.3
D	6.7	2.1
C	6.6	0.0
O	5.8	0.0
N	9.4	0.5
S	2.8	0.0

The Protein Crystallography Station at LANSCE

Team:

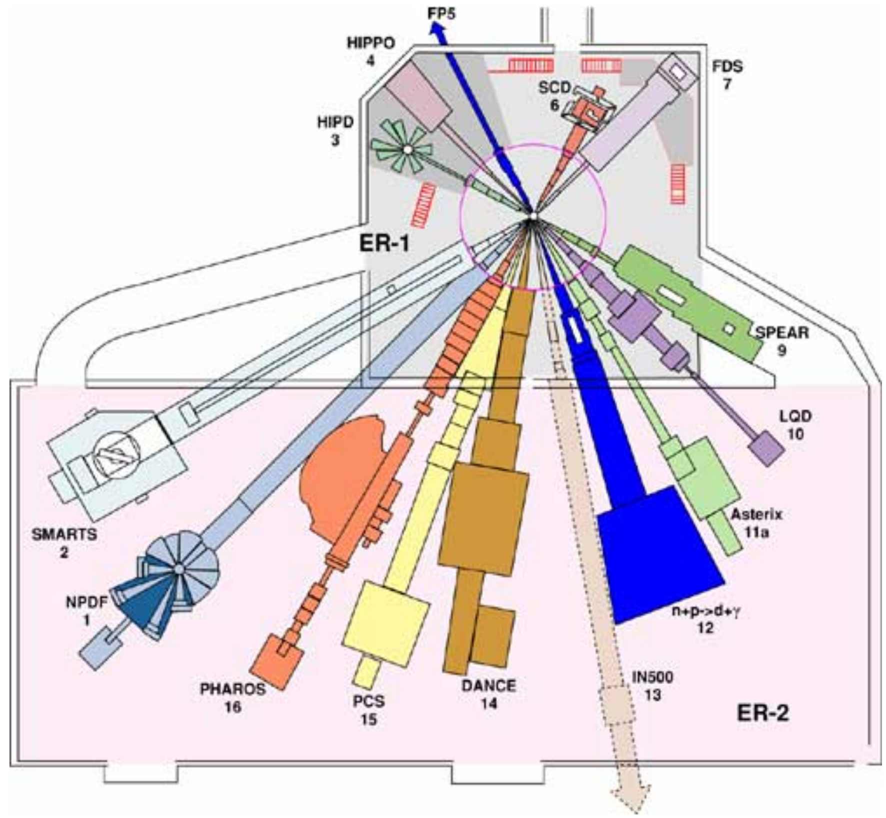
PI: Cliff Unkefer

Instrument Scientist: Zoe Fisher

Technologist: Mary Jo Waltman

Postdocs: Oksana Gerlits, John-Paul Bacik, Javier Gonzalez

Senior Advisor: Benno Schoenborn



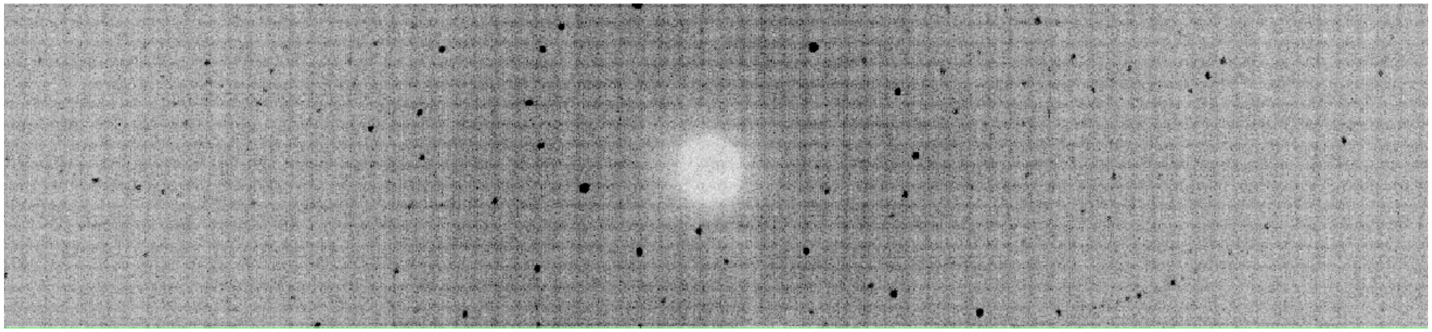
Neutron Xtallography: Pros & Cons

☺

- No radiation damage
- Elucidation of *all* H atom positions
- Protonation states, H-bonds and solvent structures (OH^-, H_2O, H_3O^+, $H_5O_2^+$ …)
- Information obtained from deuterium exchange (solvent accessibility, dynamics)
- Still in development

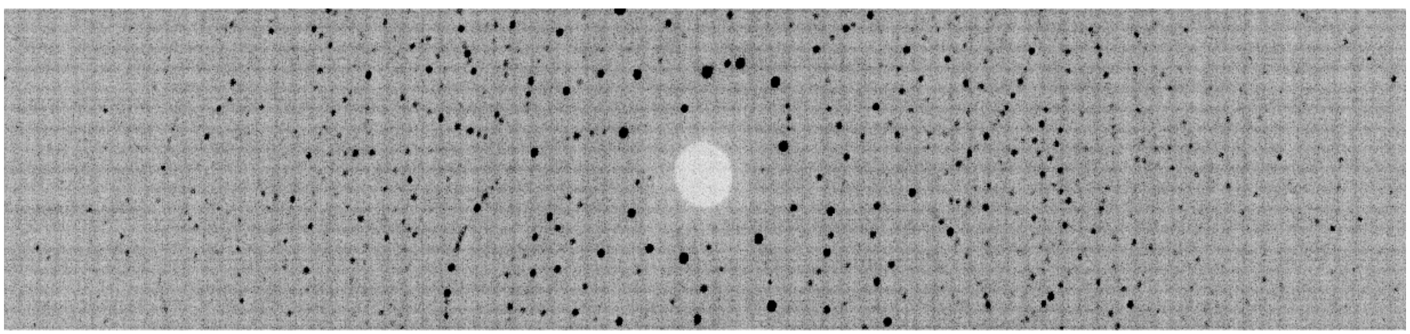
☹

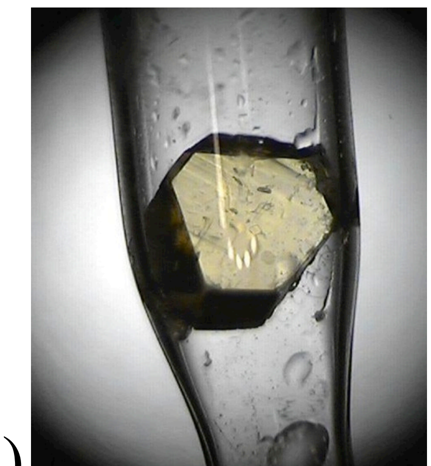
- Needs neutrons ($$$)
- Usually at room temperature
- Sample (per-) deuteration
- Crystal size of mm^3
- Available neutron sources are few and weak (long data collection, poor statistics)
- Diffraction quality and unit cell limitations
- Still in development



~ 1 mm^3, 32 hrs exposure (2.0 Å resolution)

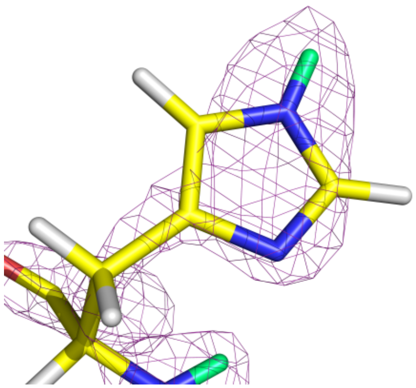
Bigger is better



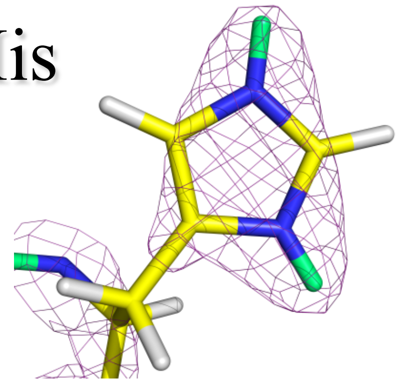


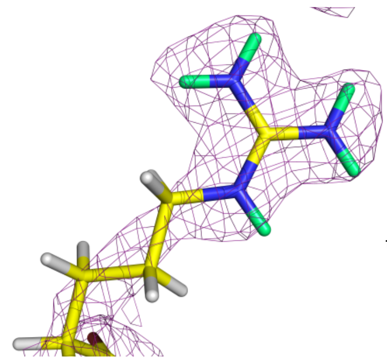
~ 30 mm^3, 12 hrs exposure (1.8 Å resolution)

Typical nuclear density maps

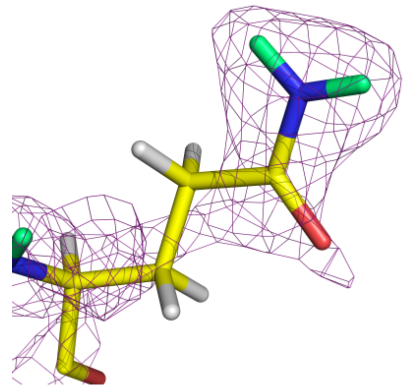


His

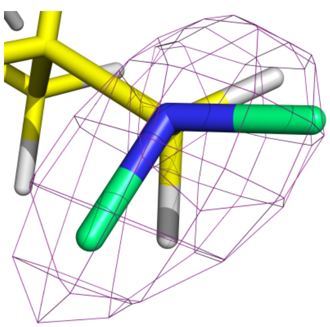


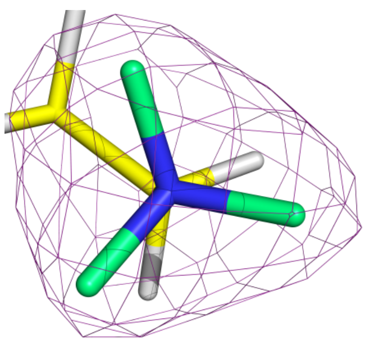


Arg

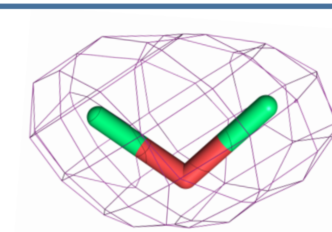


Gln





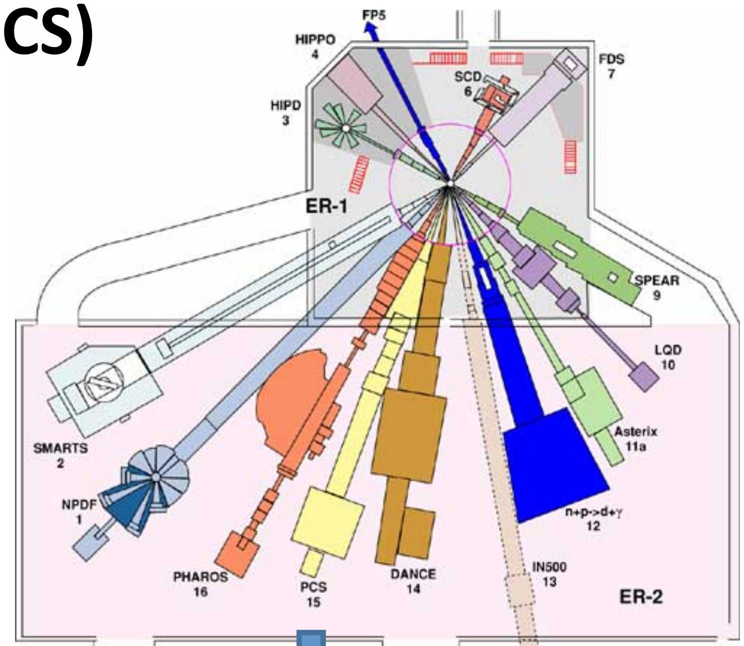
Lys

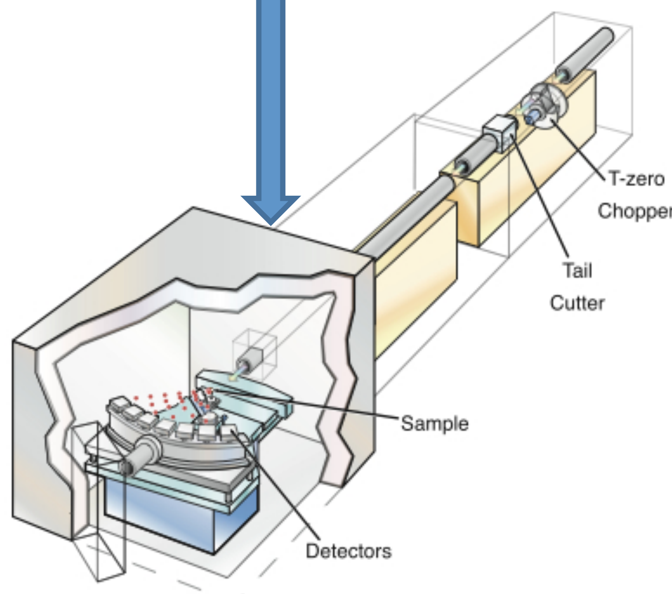


D_2O

Protein Crystallography Station (PCS)

- PCS is a high performance, state-of-the-art neutron crystallography beamline (DOE-OBER funded)
- Unique - 1st detector of its kind built at a spallation source
- User program and instrument commissioned in '02
- Accommodate 25-50 users a year, 15-25 proposals a year

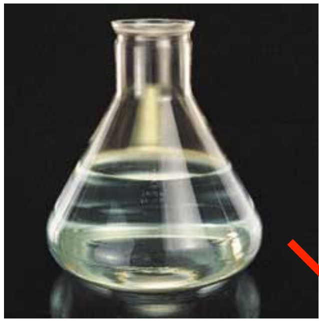




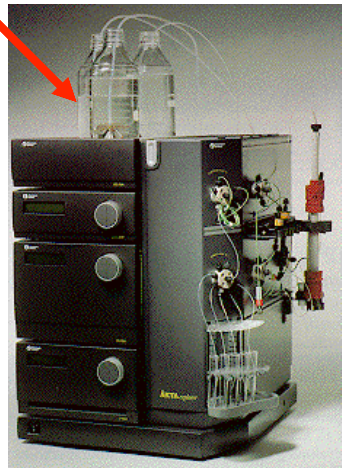
Protein Crystallography Station (PCS)

- ^3He position-sensitive neutron detector (BNL)
- 120° coverage
- K-circle goniometer
- Hüber motors
- Usable wavelength range 0.6 – 7.0 Å (TOF, Laue)
- > 80% counting efficiency (very sensitive)
- Relatively insensitive to H background (can collect H/D samples with great success)
- Smallest done here so far 0.3 mm^3, most are in the 1 – 7 mm^3 range

Support for Users

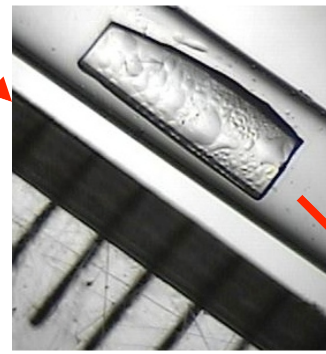


BDL (Bio-Deuteration Laboratory)

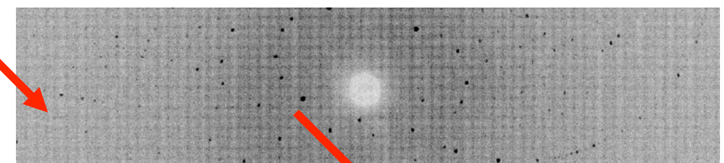


PPCL (Protein Purification and Crystallization Lab)

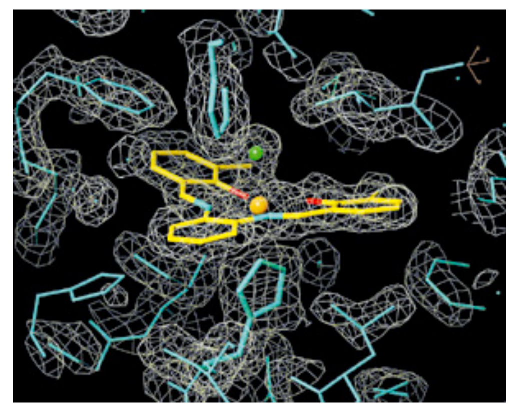
Grow large crystals of target proteins



PCS - Collect and process neutron and X-ray diffraction data

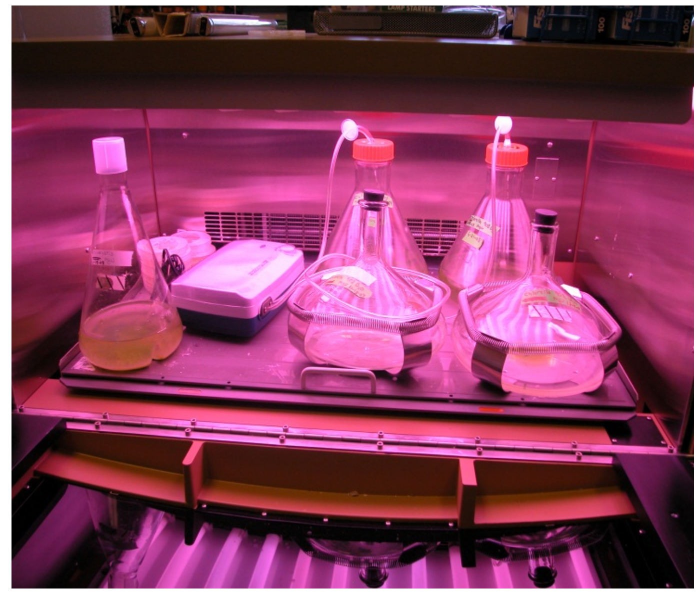


Support for using joing neutron and X-ray software



PCS Support Labs

- Protein purification facilities
- Protein crystallization facilities
- Liquid handling robot
- In-house Rigaku HighFlux X-ray home source
- Biodeuteration lab
- Algae farm to make Altone
- **Data processing software (*n*CNS, phenix.refine)**



PCS Support Labs

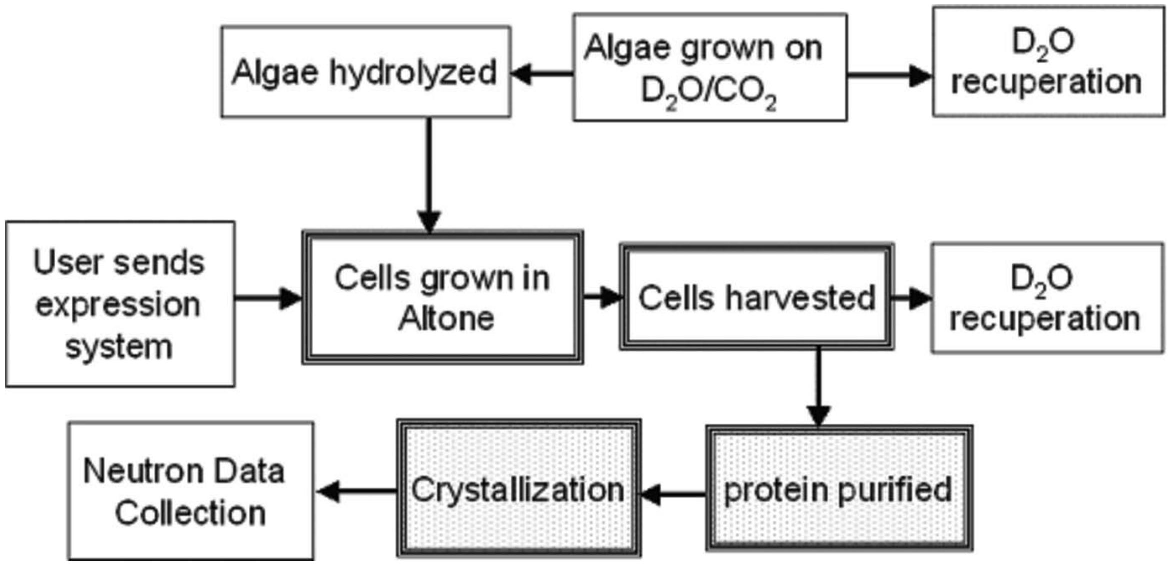


Figure 3
A schematic representation of the process used for perdeuterating proteins at the BDL at Los Alamos National Laboratory (LANL). A bacterial culture medium, which we designate Altone, is made from the hydrolyzate of algae (*e.g. Scenedesmus obliquus*) grown in D_2O and used for protein expression in *E. coli*. Steps represented by framed boxes can be performed robotically at LANL. Steps represented by shaded boxes can be performed either at LANL or the user's home laboratory.

Paul Langan *et al. J. Synchrotron Rad.* (2008). **15**, 215–218

Neutron structures determined at the PCS

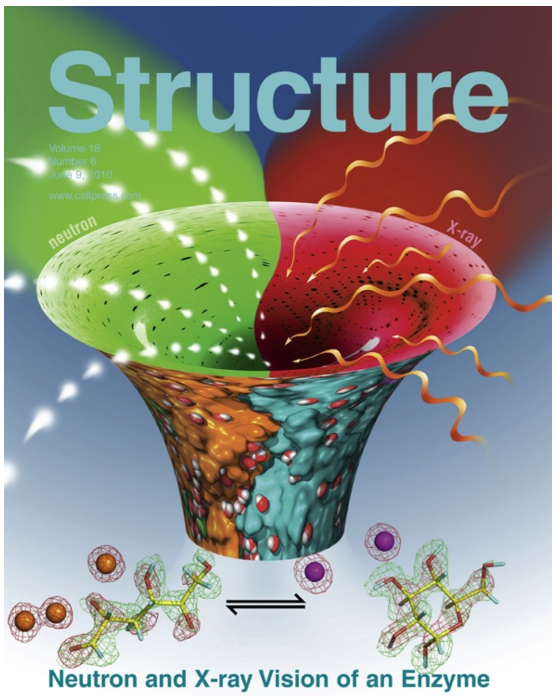
Macromolecule	Space group	Resolution, Å	Completeness, %	Crystal size, mm^3	Data Collection days	PDB ID	Citation
Crambin	$P2_1$	1.1	78.8	~5	21	---	Chen *et al.*, 2012
Rubredoxin, W3Y mutant	$P2_12_12_1$	2.1	72	~2	5	---	Li *et al.*, 2004
E. coli DHFR/Methotrexate	$P6_1$	2.2	80	0.3	23	2INQ	Bennett *et al.*, 2006
XI-2Co^{2+}	$I222$	1.8	78	8	19	2GVE	Katz *et al.*, 2006
Z-DNA, d(CGCGCG)	$P2_12_12_1$	1.6	62	0.7	18	---	Langan *et al.*, 2006
PYP	$P6_3$	2.5	89	0.8	14	2QWS	Fisher *et al.*, 2007
Endothiapepsin/*gem-diol* inhibitor	$P2_1$	1.8	80	2.7	37	2VS2	Tuan *et al.*, 2007; Coates *et al.*, 2008
Deoxy human hemoglobin (HbA)	$P2_1$	2.0	87	20	18	3KMF	Kovalevsky *et al.*, 2008 & 2010
XI-2Mg^{2+}-D-xylulose	$I222$	2.2	89	4	37	3CWH	Kovalevsky *et al.*, 2008
Carbonic anhydrase (pH=10)	$P2_1$	2.0	85	1.2	55	3KKX	Fisher *et al.*, 2009; Fisher *et al.*, 2010
DFPase	$P2_12_12_1$	2.2	82	0.43	37	3BYC	Blum *et al.*, 2009
Amicyanin	$P2_1$	1.8	68	2.6	21	3L45	Sukumar et al., 2005; Sukumar *et al.*, 2010
XI-2Ni^{2+}-D-glucose	$I222$	1.8	84	15	20	3KCO	Kovalevsky *et al.*, 2010
Equine CNmet hemoglobin	$C2$	2.0	80	10	28	---	Kovalevsky *et al.*, 2010
Human glycosyltransferase	$C222_1$	2.5	84	0.4	14	---	Schuman *et al.*, 2011
Bovine γ-chymotrypsin	$P4_22_12$	2.0	85	1.5	12	---	Lazar *et al.*, 2011
apo-XI (pH=5.9)	$I222$	2.0	87	9	10	3QZA	Kovalevsky *et al.*, 2011
Xylanase	$P2_12_12_1$	1.8	88	7	15	---	Kovalevsky *et al.*, 2011
Z-DNA	$P2_12_12_1$	1.4	-	-	-	3QBA	Fenn *et al.*, 2011
HCA II + acetazolamide	$P2_1$	2.0	85	2.0	22	---	Aggarwal *et al.* 2012 in preparation
HCA II (pH=7.8)	$P2_1$	2.0	85	1.7	20	3TMJ	Fisher *et al.*, 2011

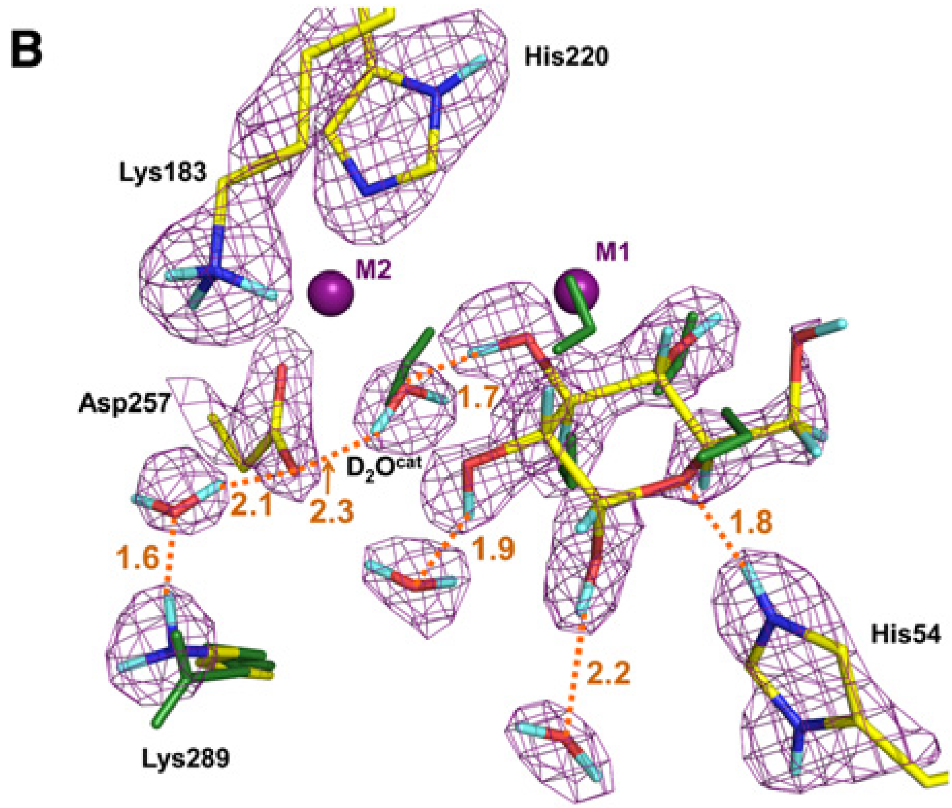
Publications

Structure *18*, 688–699, June 9, 2010

Metal Ion Roles and the Movement of Hydrogen during Reaction Catalyzed by D-Xylose Isomerase: A Joint X-Ray and Neutron Diffraction Study

Andrey Y. Kovalevsky,[1,*] Leif Hanson,[2] S. Zoe Fisher,[1] Marat Mustyakimov,[1] Sax A. Mason,[3] V. Trevor Forsyth,[3,4] Matthew P. Blakeley,[3] David. A. Keen,[5] Trixie Wagner,[1,7] H.L. Carrell,[6] Amy K. Katz,[6] Jenny P. Glusker,[6] and Paul Langan[1,2,*]



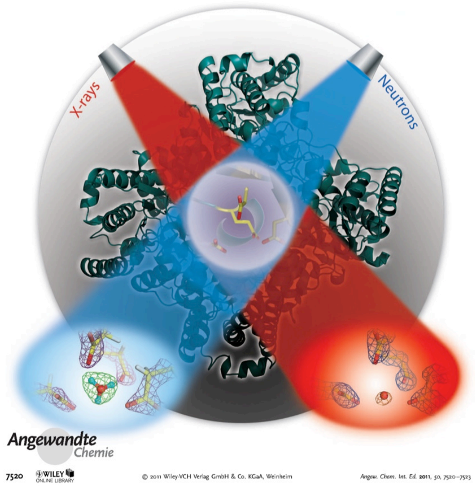


Los Alamos NATIONAL LABORATORY EST. 1943

UNCLASSIFIED

NNSA

Publications



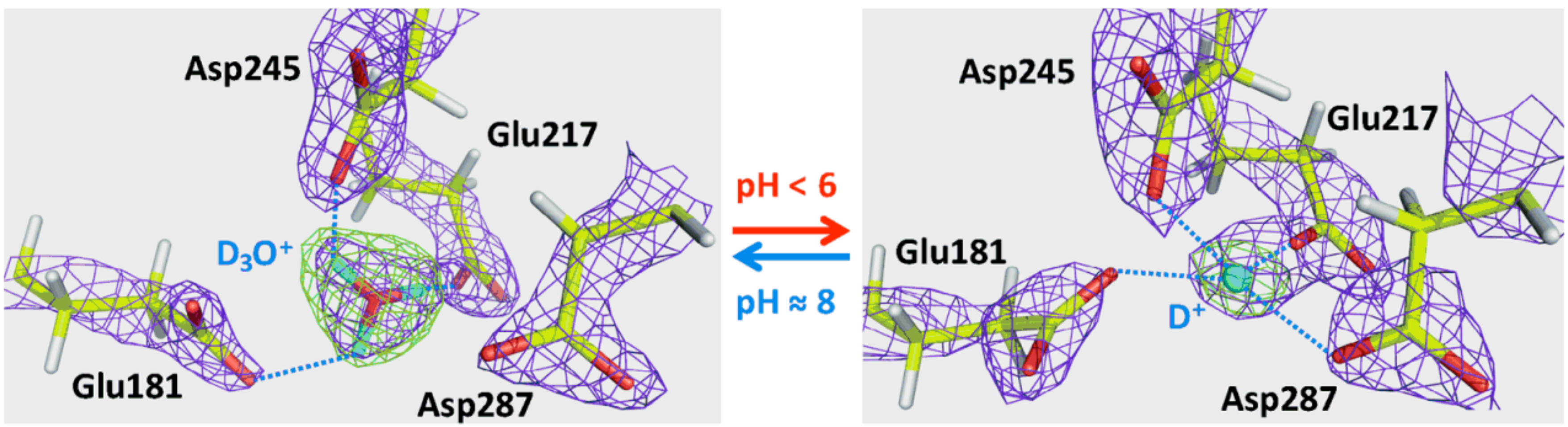
Angew. Chem. Int. Ed. **2011**, 50, 7520–7523

DOI: 10.1002/anie.201101753

X-ray/Neutron Crystallography

Identification of the Elusive Hydronium Ion Exchanging Roles with a Proton in an Enzyme at Lower pH Values**

Andrey Y. Kovalevsky, B. L. Hanson, S. A. Mason, T. Yoshida, S. Z. Fisher, M. Mustyakimov, V. T. Forsyth, M. P. Blakeley, D. A. Keen, and Paul Langan**



Publications

J. Am. Chem. Soc. 2012, 134, 14726–14729

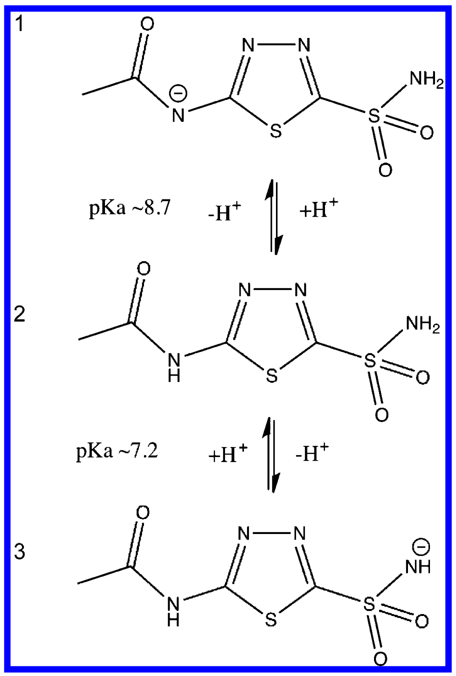
Neutron Diffraction of Acetazolamide-Bound Human Carbonic Anhydrase II Reveals Atomic Details of Drug Binding

S. Zoë Fisher,[†] Mayank Aggarwal,[‡] Andrey Y. Kovalevsky,[†] David N. Silverman,[§] and Robert McKenna*[,‡]

[†]Bioscience Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545, United States

[‡]Department of Biochemistry and Molecular Biology, University of Florida, P.O. Box 100245, Gainesville, Florida 32610, United States

[§]Department of Pharmacology and Therapeutics, University of Florida, P.O. Box 100247, Gainesville, Florida 32610, United States



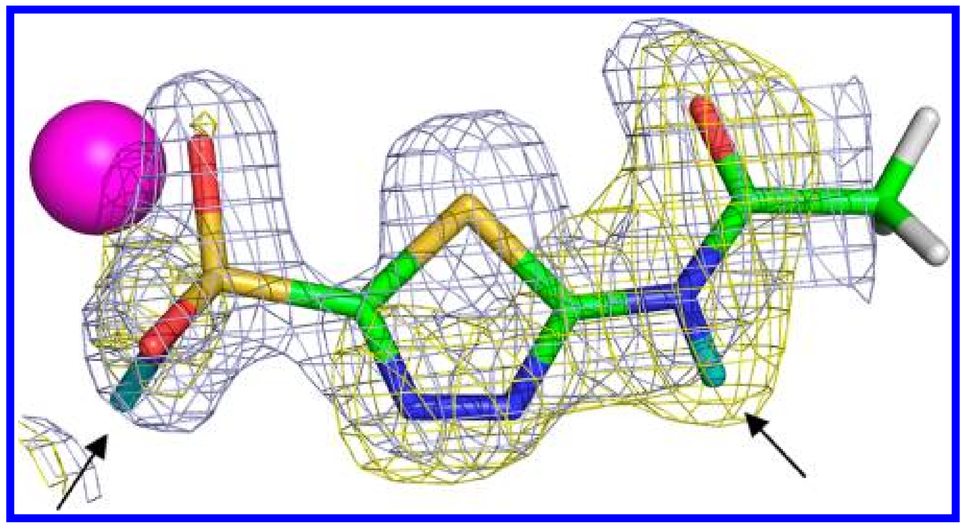


Figure 2. Stick representation of AZM bound to HCA II. Zinc is shown as a magenta sphere, D atoms are in cyan (indicated by arrows), and H atoms in white (positions calculated). The nuclear density map is shown in yellow and is contoured at 1.5 σ, and the electron density map is shown in blue and is contoured at 2.0 σ.

Publications

PNAS | **September 18, 2012** | vol. 109 | no. 38 | **15301–15306**

Direct observation of hydrogen atom dynamics and interactions by ultrahigh resolution neutron protein crystallography

Julian C.-H. Chen[a,1], B. Leif Hanson[a], S. Zoë Fisher[b], Paul Langan[a,c], and Andrey Y. Kovalevsky[b]

[a]Department of Chemistry, University of Toledo, Toledo, OH 43606; [b]Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM 87544; and [c]Biology and Soft Matter Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831

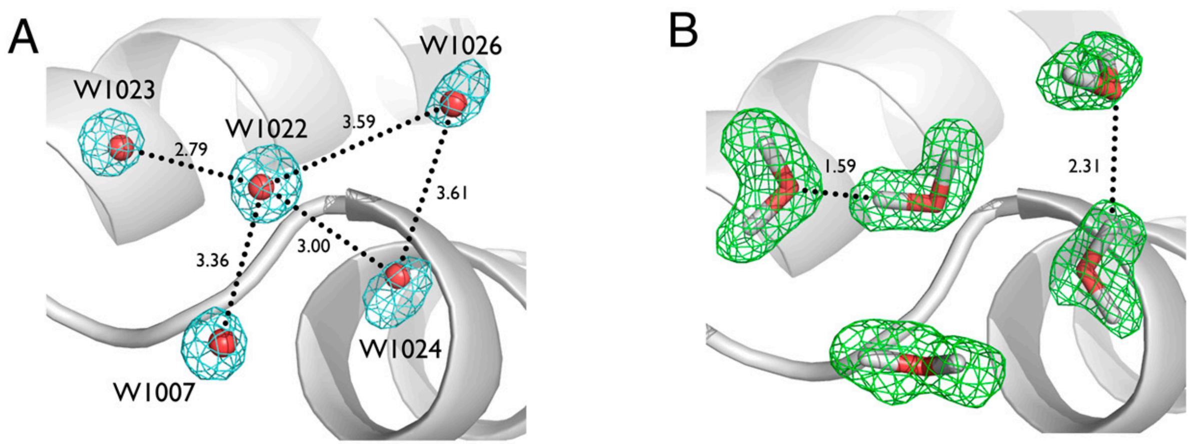


Fig. 5. Resolving a hydrogen bond network. (*A*) 0.85 Å X-ray electron density map showing potential H bond partners for W1022. (*B*) 1.1 Å nuclear density map showing that W1022 interacts with W1023, but not W1007, W1024, or W1026. (*C*) Hydration environment around Y44. A potential O—D…π interaction is highlighted involving W1023. Distances are indicated in Å.

Take-home messages

- Neutron crystallography has the potential to describe molecular details untraceable through ultra-high resolution X-ray crystallography
- Different isotopes scatter neutrons differently (e.g. $^{1}H/^{2}H$ exchange), increasing the experimental possibilities
- Accurate description of protein-ligand interactions is particularly useful for Structural Enzymology
- The User Program at PCS offers a full support for protein neutron crystallography studies